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NUTRITION REVIEWS

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BIASED CRITICISM OF FLUORIDATION

Public health measures of any sort should be able to stand up to searching criticism; in fact it is a matter of great interest to the public that such criticism should exist and should be met. It is only reasonable to expect, however, that the criticism shall be fair-minded, shall present mistakes or inadequacies in their proper relationship to correct and adequate accomplishments, and shall not present conclusions to the public which are unwarranted by the evidence. The occasion for these remarks is the publication of an 83-page booklet, *Fluoridation, Errors and Omissions in Experimental Trials* by Philip R. N. Sutton (Melbourne University Press, Victoria, Australia, 1959, available through Cambridge University Press, New York).

Dr. Sutton, a Senior Research Fellow at the University of Melbourne Dental School and apparently without field experience in the epidemiological study of fluoridation, has devoted himself to a critical analysis of the printed reports of the long-term pilot studies of water fluoridation made in Grand Rapids, Michigan; Evanston, Illinois; Brantford, Ontario; and Newburgh, New York. The result is pure destructive criticism. A few errors and omissions are pointed out which might have been avoided. There are two or three valuable suggestions for future pilot studies of a similar nature. The bulk of Sutton's material, however, comprises criticism of a minor or irrelevant nature with no attempt to appraise the large positive accomplishments and extraordinarily comparable results of the four fluoridation projects. On frequent occasions he makes the mistake of trying to answer with a categorical "yes" or "no" questions which are far too complex for such black-and-white treatment. Such thinking also finds its way into his final conclusion that, "The sound basis on which the efficacy of the public health measure must be assessed is not provided by these . . . crucial trials."

Sutton's criticisms deal chiefly with four of the more important aspects of project design: adequacy of controls, randomization of sampling procedure, adjustment of sample size to compensate for variability in caries attack rate, and variability or bias of examiner. Each of these deserves separate attention.

In discussing requirements for a control, Sutton adopts the position that the control city should be "comparable in all respects" to that where fluoride is being added. Thus he criticizes the selection of Kingston as a control for Newburgh because there are differences in calcium, magnesium and hardness of the water between the two cities. It does not seem to concern him that only with the third of these characteristics is there even a hint of evidence that it may be related to caries attack rate, nor that even if a relationship were known to exist in all three characteristics the matter would be irrelevant if the balance of other factors brought it to pass that initial caries rates were roughly similar in the two cities before fluoridation, which was indeed the case.

He expresses similar concern over the possible climatic and other differences between pilot and control cities, harking back to the caution quite correctly shown by H. T. Dean and other early students of the caries-fluorine hypothesis, when attempting to compare different endemic levels of dental caries in cities where fluoride level also differed. At this point, of course, it was very necessary to realize the possible existence of causative factors other than the fluoride in the water. Such caution is not necessary where the control city passes all reasonable tests for comparability including that of a similar initial caries rate, and the experiment involves a known environ-

mental change in the study city. Strict observance of Sutton's desires on selection of a control city would make epidemiological study virtually impossible.

Another criticism which deals with adequacy of control concerns the number of examinations made in each control city. Minor fluctuations in caries rate in the various control cities are made a matter of criticism even though these fluctuations do not contradict the general trend toward steady caries rates in the control cities and dropping caries rates in the pilot cities. Muskegon, Michigan, is criticized as a control city because fluoridation was instituted there three years before the end of the Grand Rapids study period. No consideration is given to the fact that within three years the demonstrable results of fluoridation are extremely small and are confined to certain very young age groups, nor to the fact that what influence may occur is in the direction of reducing differences in caries rate from a previously fluoridated city. Thus the differences which remain achieve added significance.

Sutton apparently has a fixation on the subject of random sampling, and is not familiar with the principle of stratified sampling designed to handle situations where randomization alone could produce biased results. Thus he criticizes the selection of representative schools in the large city of Grand Rapids and the examination of "almost all schools" in Muskegon, a smaller city. Carrying this thought further, he criticizes the Evanston study because white children in the control area (Oak Park) were compared with white children in Evanston instead of white and negro (Negroes have a different caries rate). The randomization Sutton suggests would, therefore, have introduced errors instead of eliminating them from the studies.

On the control of variability in dental caries experience, Sutton assumes no planning to have occurred. He does not realize the rather steady pattern of such variability and the fact that it can be overcome by adequate sample size. With Muskegon, for instance, he draws attention to the fact that 12 of the samples used contained fewer than 20 children each. He does not state that there were 130 samples examined in Muskegon, all told, over a period of ten years. Seventy-nine of them contained over 100 children, and 57 of them over 200 children. The large size of the average sample in this instance is very clear evidence of a good approach to the question of variability.

It seems apparent from Sutton's comments upon examiner variability that nothing will satisfy him except the continuous employment of a very small number of examiners for the entire ten-year period of study. It seems to mean nothing to him that the studies were under continuous supervision and, if examiners changed, the new ones were oriented and standardized by the supervisor. He considers it a possible source of bias that the examiners knew in which city they were operating. In this connection, he makes the good, though difficult, suggestion that such bias might be eliminated by transporting to a common examination center children from both cities so that the residence of the child might be unknown to the examiner. He shows bias of his own, however, in refusing to credit the fact that x-rays of Newburgh and Kingston children, randomized by code number, showed results of the same order as those made by the clinical method in each city.

His most striking examples of variability are presented in connection with children from six to eight years of age, when their deciduous teeth are exfoliating, and caries in the permanent teeth is of the pit-and-fissure type which makes determination of time of onset so difficult. He neglects the much more consistent values seen among older children toward the latter part of the study period, a finding so consistently duplicated from one study to another.

It is natural that Sutton's book should have drawn comment from the principal investigators in the four fluoridation study areas which are criticized. Some of these comments are given herewith from letters to Dr. Kenneth Adamson, President of the Australian Dental Association.

Grand Rapids Study

Dr. F. A. Arnold, Jr., Director of the National Institute of Dental Research and principal investigator at Grand Rapids, writes in part as follows (*Arnold to Adamson, October 16, 1959*):

"He (Sutton) overlooks the fact that one examiner has been with the study throughout. If we used his findings, we would come up with the same general result. Also, we could call attention to the fact that two more of the four examiners used throughout the first ten years of the study started examining during the third year of the study and have participated each year since.

"He criticizes our selecting samples by school grade. If he would realize it, and probably he does, this strengthens the study. In the first place, this gives us a random sample. Also, it permitted us to examine all the children of a grade without the examiners knowing whether the child belonged to the 'continuous resident' group or not.

"Although he did not publish his material until 1959, he (apparently intentionally) overlooked the report of the tenth year of the study which appeared in 1956. As was originally planned, it was this year that we obtained 'complete' age groups of adequate size. The planning of the study and the analysis of the data were done by a group of people all of whom are more knowledgeable in this field of research than is Dr. Sutton."

Newburgh Study

Dr. David B. Ast, Director, Bureau of Dental Health of the New York State Department of Health, makes the following comments (*Ast to Adamson, March 3, 1960*):

"Sutton criticizes the comparability of data among the four studies because in Newburgh and Kingston we used the rate based on DMF per 100 erupted permanent teeth instead of DMF per child. We explained why we used the permanent tooth population as the universe to be considered. However, in order to make our data comparable to other study data, in the reports for 1953–54 and 1954–55, the Newburgh-Kingston data were given both ways—DMF per 100 teeth, and DMF per child. What is significant and had eluded Sutton is the fact that the percentage differences in Newburgh and Kingston were almost the same for both methods used.

"Another criticism made is that base-line data were collected in Kingston a year after the Newburgh survey. I can't believe Sutton really believes this to be a valid criticism. He must be, or should be aware of the fact that caries is not an acute disease of short duration, but a slowly developing one . . . The base-line data in Newburgh and Kingston based on the examination of all of the school children age six to 12 in both cities were almost identical. All of the examinations were made by one examiner. Could Sutton really believe that the DMF rate of 20.8 for Kingston, and 21.0 for Newburgh could have been significantly different if both examinations were made exactly at the same time? . . . This type of criticism questions not the research but the professional acumen of the critic."

The Evanston Study

J. R. Blayney, Director of the Evanston Dental Caries Study, comments thus (*Blayney to Adamson, November 23, 1959*):

"Dr. Sutton . . . states, 'The arbitrary selection of the data which is then termed 'representative', instead of making the ultimate findings to be considered valid and reliable, would render a report based on this selective data unfit for serious consider-

ation.' We feel that this type of criticism is unworthy of the scientific stature and dignity of the University of Melbourne and would tend to imply that the rather reasonable separation of white and negro, public and parochial school children, for the purpose of comparing like with like, is an 'arbitrary selection' making the 'data unfit for serious consideration' and that the entire report hinges only on this preselected data. We have gathered no secret or concealed data . . .

"Dr. Sutton was concerned that the control group was not examined annually. Neither we nor our advisors could see a reason to require an examination of the control group other than at the beginning and near the close of the study. This provides the base-line from which to measure the trend of the dental caries rate during the time interval (1947–1956). Should the rate in the last examination (1956) deviate materially from that of the initial base-line period (1947), that figure could be used as a correction factor on the Evanston findings.

"It is true that a discrepancy in figures published in our paper XVI, Table I, and in paper XVIII, Table I, are at variance. This is due to the operator of the tabulating machine providing the wrong figures for the number of seven- and eight-year-old children examined. This error was discovered after manuscript XVI was in press and therefore the corrections could only be made manually in the reprints supplied to readers who requested them."

The Brantford Studies

R. M. Grainger of the University of Toronto writes (*Grainger to Adamson, December 4, 1959*):

"To be brief, the writer is correct if he is making the point that the fluoridation studies are not flawless examples of scientific literature. He is also correct if he implies that the exact amount of protection against tooth decay from fluoridation cannot be pinpointed and that its protection may not be equal for all populations or under all circumstances. He is not correct if he implies that the slips in arithmetic, typographical errors, unsophisticated statistical analyses, minor inconsistencies between preliminary and final reports or between observer teams, which he has unearthed, throw any serious doubt on the corroborative conclusions of the water-fluoridation experiments or on the public health value of the procedure.

"Page 38, middle paragraph, re late commencement of National Health and Welfare Study and detection of caries protection for young individuals born prior to commencement of fluoridation. Despite the fact that the Department of National Health and Welfare began its control study nearly three years after fluoridation began, much worthwhile information was obtained and the effect of late commencement, if any, was to result in underestimation of the fluoride protection.

"Page 44, last paragraph, re differences in reported rates between examiners. Different examiners give characteristically higher or lower rates upon examining the same individuals due to differences in skill, training and physical condition. Thus the differences quoted are no reflection on the design of the experiment or the care taken in the work. The strength of the double examinations comes through corroboration of caries trends in Brantford over the years and not through interchangeability of data."

If Dr. Sutton had visited with an open mind such cities as Newburgh and Kingston, as this writer has done recently, he would see such obvious differences in children's mouths even on throat-stick inspection that he would stop looking for "slips in arithmetic, typographical errors, unsophisticated statistical analyses, and minor inconsistencies between preliminary and final reports or between observer teams." The same would also be true if Sutton had given a fair share of his attention to the

great mass of positive and unimpeachable findings of the various studies. Going even further, he might have included in his reasoning the great mass of supporting data on benefits of fluoridation available for studies in the endemic fluoride areas and now, increasingly, in fluoridating cities other than the four original ones.

Sutton was technically within his rights in confining his volume to destructive criticism, but the tragedy is that his work will be read by a public often unable to appreciate its defects or learn in detail the other side of the case. Worst of all the book will be used by unprincipled agitators to arouse fear in citizens' groups where fluoridation is up for community decision.

D. J. Galagan of the U.S. Public Health Service makes remarks as follows (*Galagan to Adamson, November 27, 1959*) concerning the effect of the book upon Australian children (and this applies to some degree also to American children):

"I am sure that the report, seemingly published in good faith . . . will delay further the fluoridation of municipal water supplies in Australia. The many thousands of Australian children who will needlessly develop dental caries, and suffer unnecessarily the pains of toothache and infection, will have Dr. Sutton to thank. I think it would weigh heavily on his conscience."

James M. Dunning, D.D.S., M.P.H.
Harvard School of Dental Medicine
Boston, Massachusetts

LONGITUDINAL STUDIES OF CHILD HEALTH AND DEVELOPMENT

Long-term studies of skeletal maturation, height, weight, protein and caloric intake and illness experience of 134 children emphasize individual variability at each age period but reveal individual patterns of performance.

A number of long-term and multi-disciplinary studies of growth and development of children were begun about 1929 or the years immediately following. Reports of progress from these have appeared from time to time, but these reports have usually dealt with findings in single areas of research and for a limited age period. Some of these research projects are continuing in full or extended operations, notably one in Denver under the direction of A. Washburn and one at the Fels Institute in Ohio under L. W. Sontag. Others are operating in a modified form or have been terminated (H. C. Stuart, *Pediatrics* **24,** *701 (1959)*; K. Simmons and T. W. Todd, *Growth* **2,** *93 (1938)*; H. W. Jones, *J. Consult. Psychol.* **3,** *157, 177 (1939)*).

The Longitudinal Studies of Child Health and Development, carried out by the Department of Maternal and Child Health at the Harvard School of Public Health, were started by H. C. Stuart in 1929 and conducted under his direction through 1956, at which time study of the last child enrolled had been completed. The data collected in this project include observations during pregnancy and periodic observations of growth and development from birth to 18 years of 134 children equally divided between boys and girls. Early phases of this work are described in a monograph published in 1939 after the last child had been enrolled (Stuart *et al., Monograph of Soc. for Res. in Child Devel.* **4,** *Serial 20, No. 1 (1939)*).

Recently, a major series of reports on these longitudinal studies have appeared as a supplement to *Pediatrics* (Stuart *et al., Pediatrics* **24,** *875, 886, 904, 922, 941 (1959)*).

In introducing this series of papers, Stuart *et al.* state that variability in the growth and developmental progress of children is related to their health. Although growth patterns and developmental progress are determined in large measure by hereditary factors, these intrinsic factors may be modified by disease or other environmental

changes. This modification of patterns helps to separate the influences of heredity from those of environment. Nevertheless, individual differences in response to environmental factors make differentiation between "normal variations" and changes brought about by disease difficult. It is also emphasized that all of the above factors apply to the developing fetus and to the newborn infant, although our information about them is less direct. Consequently, studies of health during pregnancy are important to the understanding of growth and development throughout the whole of the growth cycle.

Since there are certain consistencies in individual growth patterns, differences between individuals deserve particular study. A number of basic concepts are inherent in the interpretation of a study of this scope. It is pointed out that there are recognizable stages in the human pattern of growth and development, each having particular health needs; thus improvement of personal health programs for children depends upon the recognition of these needs. At a given age, there is a characteristic distribution of a measurable attribute of growth and development but with differences between different groups of children. This distribution does not permit definition of normal. Moreover, individual differences in the magnitude and timing of developmental changes and in the consistency of growth patterns make individual study necessary. Knowledge of the interrelatedness between measurable attributes for individuals will reveal those attributes that are the best overall indicators of longitudinal progress.

One hundred and thirty-four children were studied. Except for six considered premature because of birth weight, these children were all full-term infants. They came from self-supporting families of Northern European stock largely of Irish extraction. Inclusion in the study group depended upon attendance at the prenatal clinic of the Boston Lying-In Hospital and willingness to participate.

The various papers in the present monograph report the patterns of skeletal development in the hand, of growth in height and weight, caloric and protein intakes, and patterns of illness experience. Data concerning interrelationships between these measurable attributes is planned for publication in the near future.

Skeletal development was assessed by comparing radiographs of the hand with the standards of W. W. Greulich and S. I. Pyle (*Radiographic Atlas of Skeletal Development of the Hand and Wrist. Stanford University Press (1950); second edition (1959)*). The mean values for the group as a whole were slightly faster than the standards for infancy, slightly slower during childhood, and slightly faster during adolescence. These deviations were representative of the group but were not statistically significant.

Some individual patterns (determined by plotting skeletal age (hand) versus chronological age) were consistently faster or slower than the standards, while others were variable. By assessing the relative rate of skeletal development the children were divided into those who had a consistent pattern during the whole 18 years of the study, those who had differing rates at two age periods, those with differing rates at three age periods, and finally those whose pattern was irregular. By assessing individual patterns as slow, moderate, or fast, 13 patterns were found out of a possible 21.

Patterns were classified as fast or slow if they changed more than one standard deviation during the age period under consideration. Twenty-seven children had a moderate rate of skeletal maturation during the whole of the growth period. Fifteen children who grew more rapidly during the second half of the growth period had had a moderate rate initially. Fourteen children had an initial slow rate and a subsequent fast rate. Eighteen children were classified as irregular. To classify the results somewhat differently it was felt that 35 children had consistent patterns, another 35 speeded

up their rate of skeletal maturation in later childhood and adolescence, 18 had irregular patterns and the rest fell into a number of different groups.

The results are presented graphically in a manner emphasizing individual variation. The great mass of observations is at first bewildering, but the reader soon grasps the system of classification. One of the more interesting findings was that a majority of the twins had very similar patterns of skeletal maturation even if they were not identical twins. Other children had very similar patterns suggesting the possibility of genetic similarity in individuals who were not related.

Accelerated patterns of skeletal maturation were seen in children who at one year of age were either delayed or advanced. It is interesting that this acceleration was greater in the children considered advanced in bone age at one year than it was in those whose bone age was relatively slow at one year. The similarities between sexes were impressive using standards which were adjusted for the more advanced bone age of girls.

It is concluded, therefore, that there is great variety in the rate of skeletal maturation in normal children. The great majority of children have patterns of bone maturation which fall into a few general categories. The most common change in pattern is acceleration between childhood and adolescence.

Data on height and weight are reported in yearly averages and ranges for the two sexes separately. The 18 years under study were divided into three periods of six years each; the total growth increment was calculated for each period and its relative magnitude assessed according to the group as a whole. The individuals falling in the extreme quartiles of the distribution were designated as slow or rapid during the six-year period, while those within the median 50 per cent were considered moderate in growth rate.

The 134 individuals in the study fell into five main groups. Those with rapid growth during the first six years of life tended to mature early, while those with slow growth during the first six years of life had a later pre-adolescent growth spurt. For those with moderate growth in the pre-school years, the time of the pre-adolescent growth spurt was variable. The correlation of growth before six years with final height at 18 years was also noted.

Similar patterns were seen for both height and weight increments and emphasize the range of variation. A number of figures in this article showing the patterns of individual growth curves against the background of the group as a whole demonstrate the range of variation better than any statistical analysis.

The caloric and protein intakes were estimated by "the Burke dietary history method." Since 66 per cent of the infants were breast-fed for varying periods during the first year of life, no data were reported for infants under the age of one year. Yearly averages from one to 18 years are given for calories and protein. There was a great deal of variation in the data, with one child having twice the intake of calories and protein as another child in the same age and sex group. In all ages the males had greater intakes than the females, except for the protein intake under six years of age. The averages were slightly higher than those of the Recommended Dietary Allowances of the National Research Council (*NAS-NRC Publication 589, Washington, D. C. (1958)*), except for the caloric intake of the girls. In comparison with other recent studies of caloric and protein intakes of American and British children, reasonably close agreement was found.

The longitudinal cumulative intakes in the various age periods to 18 years were divided into high, medium and low categories and many different patterns and relative changes with respect to age were encountered. There were no clear-cut patterns to represent the dietary habits of large numbers

of children. Although the authors made no effort to relate caloric to protein intake, a few calculations from the data suggest that the great majority of the children of all ages had protein intakes representing about 12 to 14 per cent of calories.

In dealing with the illness experience of 134 children, each followed for almost 18 years, a quantitative assessment was extremely difficult. An elaborate classification of illnesses as to number, severity and duration was adopted and the available historical data were all reviewed by one individual. Numerical scores for illness experience were evolved which ranged from 1 to 25 for single illnesses.

As might be expected, the boys had a greater number of illnesses than did the girls although the scores for severity of illness were only slightly higher in the males. Both the number of illnesses and the severity scores were highest in the pre-school ages and lowest in the adolescents. In order to evaluate possible patterns of illness experience within the group, classifications were made based on score and frequency of illnesses. This resulted in the characterization of 14 separate patterns.

This monograph represents only a small portion of the data collected in the long-term study of these 134 children. The techniques employed to develop objective means of assessing long-term patterns of growth, development, dietary intake and illness experience are novel and constitute a valuable contribution to methodology. The presentation of such a mass of information without any attempt at intercorrelation of the various parameters measured is extremely tantalizing to the reader. It is hoped that the relationships which might exist between illness experience, growth, maturation and nutrition have been studied as extensively as the basic observations.

HOOKWORM INFESTATION AND ABSORPTION

Severe hookworm infestation has no effect on calorie absorption, but produces a slight reduction in the absorption of nitrogen; mild infestation has no effect on either calorie or nitrogen absorption.

Individuals who are heavily infested with hookworms are usually anemic. The anemia is frequently associated with the loss of blood from the intestinal tract. Until recently it was believed that the intestinal hemorrhages with the accompanying loss of blood were entirely responsible for the poor nutritional condition of the hookworm-infested patients (*Nutrition Reviews* **6,** *181 (1948)*).

S. J. Darke (*Brit. J. Nutrition* **13,** *278 (1959)*) has shown that natives in Tanganyika who have a high count of hookworm eggs in their stools show a reduction in the absorption of dietary nitrogen. She was led to this study by the observation that the stools from anemic patients who were free from or only mildly infested with hookworms had 54.6 ± 2.7 mg. of nitrogen per gram of dry material, whereas the stools from patients with heavy hookworm infestation had 61.8 ± 1.7 mg. There was no difference in the energy contents of the stool. On the basis of these observations, the following studies were carried out.

Eight subjects for the study were from Kampala while one was a patient in the metabolic ward of the East African Institute for Medical Research. The subjects included two children, one of whom was six years old and weighed 15 kg. while the other was 12 years old and weighed 20 kg. The other subjects were adults whose ages were unknown.

All patients had occult blood in their stools and had large numbers of hookworms in their intestines. The stools passed during the 36 hours following a deworming dose of tetrachlorethylene and magnesium sulfate

contained, by actual isolation, from 416 to 2839 worms. The low value was for the six-year-old child, however, while most of the subjects passed more than 1000 worms. These counts were obviously minimal values. All subjects were severely anemic as shown by hemoglobin levels ranging from 30 to 46 per cent.

Balance studies were carried out on each subject while on the hospital diet. The food consisted of maize porridge made with water and a little milk, bread, cooked bananas, groundnut soup and either rice soup plus meat (three times a week) or sweet potatoes plus beans (four times a week). Unfortunately, the length of the balance periods was not given.

The percentage of calories absorbed as determined by bomb calorimetry of the diet and stool, ranged from 76.5 to 90.5 per cent with a mean of 86.1 ± 1.3. The intakes ranged from 1659 to 2362 calories except for the six-year-old, who received 800 calories. A week or more after deworming, the balance studies were repeated on three of the subjects and were also carried out on two other natives who were worm-free. In the absence of worms, the subjects absorbed an average of 90.0 per cent of the dietary calories. There was no significant difference in the absorption of calories between the group heavily infested with worms and the one that was worm-free.

The absorption of nitrogen in the patients heavily infested with hookworms ranged from 41.1 to 78.4 per cent, with an average of 62.5 (S.E. = 3.1). The absorption of nitrogen in the worm-free patients ranged from 67.7 to 81.6 per cent with a mean of 73.3 (S.E. = 1.9). The difference in the percentage of absorption of nitrogen (10.8) between the two groups was significant. These absorption values were secured with diets which provided a nitrogen intake for the individual subjects ranging from 5.2 to 10.9 g. per day.

Darke (*loc. cit.*) emphasized the fact that the improvement in nitrogen absorption following deworming occurred before there was any change in the anemia. On this basis, the reduction in nitrogen absorption may be considered a primary effect of the heavy hookworm infestation.

In a similar study carried out among other natives in Tanganyika, E. G. Holmes and S. J. Darke (*Brit. J. Nutrition* **13,** *266 (1959)*) found that the absorption of both calories and nitrogen was normal in patients with mild hookworm infestation. The number of hookworms isolated from the stools of some of these adult patients was usually less than 200. None of these subjects was anemic and they were presumably in better nutritional condition than the heavily infested natives studied earlier.

Eleven observations (length of balance periods and numbers of subjects not stated) were made on the absorption of calories and nitrogen before and after the subjects were dewormed. Before deworming the subjects absorbed 73.5 ± 2.0 per cent of the dietary calories while afterwards they absorbed 70.9 ± 2.2. Comparable values for nitrogen were 88.9 ± 0.7 per cent before and 87.6 ± 0.8 afterwards. The caloric intake of these subjects ranged from 2256 to 3795 calories per day, while the nitrogen intake ranged from 14.1 to 23.8 g.

The African natives who showed a very high infestation of hookworms were primarily migrant laborers who were poorly paid and consumed a poor diet. In the same area, however, there are other natives whose diet is fairly good, presumably because of an improved economic status. Although the latter are also exposed to hookworm infestation, they harbor a relatively small number of these parasites in their intestines. Nevertheless, there is little concrete evidence that the good diet affords protection against heavy parasitic infestation. Darke (*loc. cit.*) indicates that her clinical experience suggests that "it is rare to find heavy hookworm loads with severe anemia in persons whose diet is not or has not been recently grossly deficient in protein."

ASSESSMENT OF WEIGHT REDUCTION

Evaluation of reduction diets is difficult since criteria for evaluation are variable. A mathematical formula has been devised to compare data of diverse origin.

The common problem of obesity often frustrates both patient and physician. Simple restriction of calories with maintenance of an adequate diet should solve the problem. However, this program fails so often that fads, innovations and even dangerous practices flourish. One of the important factors accounting for failure is a lack of understanding of a precise estimate of normal weight, of the rate at which weight should be lost, and of the number of calories which should be eaten to achieve this hypothetical weight in a given period of time.

Much uncertainty in the minds of physicians has been created by the disparities found in published reports regarding therapy of obesity. A. R. Feinstein (*J. Chronic Diseases* **10,** *439 (1959)*) has discussed these difficulties and has suggested a means whereby a uniform measure of obesity and of the results of treatment could be applied to all investigative studies.

The first problem was to assess the degree of obesity. This has been done in many ways including measurement of the thickness of a fat fold (*Nutrition Reviews* **9,** *265 (1951)*), measurement of the specific gravity of an individual (*Ibid.* **10,** *247 (1952)*), and the use of tracer substances. One of the oldest techniques has been the use of reference tables based upon age, sex, height, and average weight of a large group of individuals. One such tabulation which came into popular use has been revised slightly throughout the years and continues to be a reasonably valid reference for persons in the United States (*Medico-actuarial Mortality Investigations, Assoc. Life Insur. Med. Dir. and Actuarial Soc. America, vol. one (1912)*). Accordingly, Feinstein accepted the tabular value as representative of the desirable weight for a given patient. He did, however, take into consideration such subjective factors as appearance, general build, and the distribution of fatty tissues.

Dietary success or failure depends upon achievement of a predetermined desirable weight selected on the basis of the above-mentioned criteria. Achievement in dietary treatment of obesity must be considered as occurring in two phases; the first, a period of active dieting when weight is lost fairly rapidly, and the second, a more prolonged period during which time the patient maintains the more desirable weight achieved. Loss of weight must occur without undue physiologic or psychologic derangements.

The author discusses a number of methods for measuring achievement of weight reduction. The simplest is a mere expression of the initial weight in pounds and the number of pounds lost after treatment. A further refinement is division of the weight lost by the duration of time. This has the disadvantage, however, of giving undue credit to extremely obese patients who might lose rapidly for a short period of time. Conversely, a less obese patient who lost weight more slowly and physiologically would seem, statistically, to be a failure. Another index of performance was described by N. Jolliffe and E. Alpert (*Med. Clin. North America* **37,** *733 (1953)*) which consisted of the ratio of actual loss to the anticipated loss times 100. This formula had the advantage of demonstrating the degree with which a patient adhered to his diet, but it did not assess the amount or relative worth of the weight loss.

Still another popular expression of the degree of obesity is in terms of per cent above desirable weight and per cent of weight loss. Some years ago this technique was introduced in a tabular form which

indicated in arbitrary figures whether a patient's dietary regimen had been successful or unsuccessful based upon the per cent weight loss achieved (M. Trulson, E. D. Walsh and E. K. Caso, *J. Am. Dietet. Assn.* **23,** *941 (1947)*). The major defect in this system was that it did not provide for an expression of the effect of surplus weight. In effect, it avoided the problem of selecting a target weight and gave undue credit to massively obese patients.

Feinstein, with an admirable penchant for mathematical formulas, established a relatively simple and logical formula which took into account each of the factors mentioned above. These included weight lost, surplus weight, target weight, initial weight, final weight (lowest achieved during dieting) and duration of time. He established what he termed a reduction coefficient (R.C.) by which he could estimate the degree of obesity of any patient. This consisted of the initial weight of the patient divided by the product of the surplus weight and the target weight, times 100. Obviously both the surplus and target weights had to be derived from the estimated figure for the patient's theoretical ideal, but practical use revealed that the variation in R.C. produced by arbitrary choice of target weight was not excessive.

The characteristics of the reduction coefficient (R.C.) are as follows: (1) The higher the initial weight of the patient, the less will be the value for the available range of variation of R.C. (2) If patients who are less than 20 per cent obese are omitted, the complete range of R.C. is from 1.3 to 5.5. For example, a patient 30 per cent overweight would have an R.C. value of less than 4.0 and a patient 40 per cent overweight would have an R.C. value of less than 3.0. (3) For the same initial weight, R.C. becomes larger as the target weight is increased. In other words, R.C. represents a reciprocal of the excess weight. Similarly he used a reduction index (R.I.) which can be obtained by multiplying R.C. by the weight loss in pounds. These formulas were then applied to clinical experience.

A series of 106 patients who were treated for obesity during a dietary study were evaluated by the above-mentioned criteria. The average weight of the group was 224 pounds and the average surplus weight was 83 pounds. By applying the criteria of success or failure as described by Trulson, Walsh and Caso (*loc. cit.*) it was found that a reduction index (R.I.) value of greater than 60 implied a fair degree of success while an R.I. value of less than 60 implied relative failure. Of his 106 patients, Feinstein found that the average R.I. value was 66 and that 47 per cent of the series had a value greater than 60. He then proceeded to apply this type of statistical evaluation to the data supplied by 13 other publications in order to compare the validity of his technique. The results expressed in tabular form would suggest an easy method for comparing the relative effectiveness of the various dietary regimens used in these 13 series of patients. At a glance, one can compare R.I. values of different groups of patients.

Such comparisons are most desirable since many diets and dietary adjuncts have been advocated by various investigators but the results of these therapeutic attempts have not been expressed in a common language which can be readily grasped. Perhaps this has contributed to the lack of confidence which many nutritionists place in the published reports of other workers not well known to them.

The formulas proposed by Feinstein seem to be simple, logical and capable of providing a universal basis for comparison of data collected by any group treating obese patients by dietary methods. Whether these formulas will prove to be universally successful, or will indeed be accepted at all, remains to be seen, but the author has applied logic and common sense in an attempt to find a common denominator which might help to solve a confusing problem.

AMINO ACID AVAILABILITY IN MAN

Beef protein scores highest in terms of nitrogen and amino acid availability when compared to milk and egg, cottage cheese, pork, milk alone, peanut butter and the control whole-egg protein.

In the third article of a series on the biological availability to human subjects of essential amino acids, J. H. Watts, C. H. Allen and L. K. Booker (*J. Am. Dietet. Assn.* **36,** *42 (1960)*) have measured the availability of the eight amino acids essential to man in diets containing whole egg and beef muscle. Previous reports from the same laboratory have described measurements of the availability to man of the eight essential amino acids and of cystine and tyrosine in diets containing whole egg, pork muscle, peanut butter, egg and milk, milk alone and cottage cheese (*J. Nutrition* **67,** *483, 497 (1959)*; *Nutrition Reviews* **17,** *268 (1959)*).

The three women and two men who were subjects of the present study were between 22 and 27 years of age and ranged in body weight from 44 to 74 kg. Four were graduate students and one a research assistant. Normal class and work schedules were followed by them during the feeding experiments. Experimental methods and design were similar to those of the earlier studies mentioned above, except that no attempt was made to correct for fecal amino acids of both endogenous and bacterial origins by feeding the low-protein basal diet in the absence of a protein test food. The low-protein regimen was omitted, since in the previous studies the fecal amino acids during feeding of the low-protein diet exceeded those measured during protein feeding. This observation led the authors to conclude that the amount of metabolic amino acids in feces varies with the type of diet and, therefore, that the inclusion of a low-protein regimen in the experimental design does not provide suitable estimates for endogenous amino acids or those synthesized in the intestine.

During a preliminary experimental period of ten days, it was determined that 0.8 g. of nitrogen (from whole-egg protein) per kg. body weight per day and 48 calories per kg. per day were required to maintain slightly positive nitrogen balance and constant body weight. Diets for each subject were held isonitrogenous and isocaloric (by sucrose supplements) during each of two subsequent ten-day experimental periods. The first four days of each period were set aside for adjustment and no urinary or fecal collections made.

The basal diet contained between 4.5 and 5.0 g. protein per day. A portion of the egg or beef protein added in the experimental periods was eaten at each of three meals. The experimental diets contained from 157 to 250 g. of beef and from 260 to 420 g. of whole egg. Total nitrogens (Kjeldahl) were measured in diets, feces and urines, and the contents of individual amino acids (microbiological assay) were measured for diets and feces. The per cent availabilities of nitrogen and of each of the essential amino acids were computed by multiplying by 100 the quotient of dietary intake less fecal excretion divided by dietary intake.

During the egg-feeding and the beef-feeding periods, mean daily nitrogen intakes were 7.01 and 7.06 g., respectively; mean daily urinary nitrogen contents were 4.24 and 5.18 g.; mean daily fecal nitrogen contents were 0.93 and 0.74 g.; mean daily nitrogen balances were +1.87 and +1.23 g.; and mean calculated percentages of available nitrogen were 86.9 and 89.6.

The dietary intake of each essential amino acid approximated or exceeded the safe intakes defined by W. C. Rose (*Current Research in the Science of Nutrition. The Nutrition Foundation* (1955)), provided an allowance was made for the methionine sparing effect of cystine. The mean calcu-

lated percentage availabilities of individual essential amino acids during egg-feeding and beef-feeding periods, respectively, were: isoleucine, 90.3 and 92.5; leucine, 90.9 and 92.8; lysine, 89.6 and 92.8; methionine, 91.4 and 90.4; phenylalanine, 90.3 and 90.2; threonine, 89.0 and 89.3; tryptophan (mean value based on three subjects only), 76.0 and 79.5; and valine, 89.8 and 90.6.

The authors interpret their data as indicating an equality of egg and beef protein with regard to the availability of essential amino acids. They point out that their data in man, while qualitatively similar to other data collected in animal investigations, do not bear out the superiority of egg protein over beef as reported for isoleucine by P. D. Deshpande, A. E. Harper, M. Collins and C. A. Elvehjem (*Arch. Biochem.* **67,** *341 (1957)*), and for lysine by B. T. Guthneck, B. A. Bennett and B. S. Schweigert (*J. Nutrition* **49,** *289 (1953)*) and by J. D. Gupta, A. M. Dakroury, Harper and Elvehjem (*Ibid.* **64,** *259 (1958)*).

Comparisons of the percentage availabilities of nitrogen and individual amino acids in egg and beef protein in this study with those for whole egg, pork muscle, peanut butter, egg and milk, milk alone and cottage cheese, calculated from data obtained in the previous studies (see above), are complicated by the fact that different subjects were used. Only male subjects were used in the preceding studies while the sexes are not indicated in the present report. Moreover, a dietary protein intake of 1.0 g. per kg. body weight per day was deemed essential for maintenance of nitrogen balance in the previous studies, whereas 0.8 g. per kg. per day was selected in the present work.

Nevertheless, if taken at face value, the mean calculated percentages of available nitrogen in previously tested proteins were, respectively: whole egg (study I), 89.0, whole egg (study II), 88.4 (compared with 86.9 per cent in the present study); pork muscle, 90.0 (compared with 89.6 per cent for beef); peanut butter, 86.5; egg and milk, 89.6; milk alone, 83.3; and cottage cheese, 89.4. Except for the value of milk alone, these values are not strikingly different from one another. Considering the wide range of individual values included in the mean and the small number of subjects involved, the significance of small differences among the various proteins is very slight.

If the whole-egg protein experiment in each study is considered as a built-in control, and if the mean calculated percentage availability for the respective whole-egg protein experiment is subtracted algebraically from the mean calculated percentage availability for the experimental period during which another protein was fed, a value is obtained for the difference in mean calculated percentage availability of nitrogen between the experimental protein and the whole-egg control. The authors' data, viewed in this way, afford the following rank order for the differences in mean calculated percentage availabilities for nitrogen: beef (+2.7), milk and egg (+1.2), cottage cheese (+1.0), pork muscle (+1.0), peanut butter (−2.5) and milk alone (three subjects only) (−5.1).

When the same consideration is given the data on amino acid availability, rank orders of the six proteins tested may be established with respect to the difference in mean calculated percentage availability of a given amino acid between the experimental protein and its whole-egg control. The ranks (one to six) of each protein with respect to each amino acid may then be summed to obtain a score (equal weight being given each amino acid). When such scores are computed, beef protein has the low (best) score of 13.5; milk and egg, 17.5; cottage cheese, 24.0; pork muscle, 25.0; milk alone, 42.0; and peanut butter, high (worst) score, 46.0. Beef protein ranks first in the availability of isoleucine, leucine, lysine, tryptophan and valine, third for methionine and threonine, and is tied for second rank with pork in respect to phenyl-

alanine. Except for methionine and phenylalanine, the essential amino acid availabilities in beef were greater than in the whole-egg control. Except for the reversed order of milk and peanut butter, the same rank order is obtained from considerations of nitrogen availability alone.

Such manipulations of the authors' data only tend to bring out more clearly what information may be gleaned from the experimental efforts the data represent. Such manipulations do not lessen, however, the underlying weakness of an experimental design which assumes no bacterial synthesis or endogenous release of amino acids (*Nutrition Reviews* **17,** *268 (1959)*). Nor does it lessen the weakness of a calculation of availability based on the difference between dietary intake and fecal excretion. Inevitably a finite quantity of intake is lost in the feeding process, and equally inevitably a finite quantity of feces is not recovered. When fecal output is subtracted from intake the two losses are additive, resulting in a falsely high value for availability. Demarcation of short fecal collections by carmine markers is subject to large error, adding greatly to intra- and interindividual variation of data based on isolated short collection periods.

In general, the present methodology used for studying amino acid availability in adult man is of questionable adequacy, no matter how carefully performed. This inadequacy involves most amino acid research in man and thus study by new, more ingenious and reliable techniques is essential.

TREATMENT OF HYPERCHOLESTEROLEMIC PATIENTS WITH NIACIN, SITOSTEROL AND SAFFLOWER OIL

Although large doses of niacin or safflower oil or sitosterol decreased serum cholesterol in man, administration of sitosterol and niacin together was more effective.

There is little doubt that coronary disease occurs more frequently in hypercholesterolemic patients than it does in normal persons (*Nutrition Reviews* **7,** *155 (1949)*), but the evidence for a cause-and-effect relationship is inadequate. The answer will have to await the development of a technique whereby the concentration of serum cholesterol can be regulated with ease so that clinicians will be able to evaluate the effect of lower levels of cholesterol upon the incidence and prognosis of coronary disease.

Of the many methods available for lowering the concentration of serum cholesterol, one has been substitution of unsaturated fats for saturated fats. Other methods include the administration of niacin, vanadium, estrogens, sitosterol or safflower oil (*Nutrition Reviews* **17,** *231, 291, 342 (1959)*).

From a preliminary study of 54 hypercholesterolemic patients, K. G. Berge, R. W. P. Achor, N. W. Barker and M. H. Power (*Am. Heart J.* **58,** *849 (1959)*) concluded that niacin in large doses could suppress the concentration of serum cholesterol for prolonged periods of time. They then attempted to assess the effectiveness of this substance in comparison with two other agents, safflower oil and sitosterol. Ten hypercholesterolemic patients were treated with 5.3 g. of niacin per day for nine to 17 months and given a placebo for three months. Then for three months they were given emulsion of safflower oil (containing 46 per cent linoleic acid glyceride), one ounce three times daily, or 20 per cent suspension of beta- and dihydro-beta-sitosterols, one ounce before each meal. Nine of the subjects received both sitosterol and niacin (4.3 g. per day) simultaneously. Determinations were made of the serum cholesterol at intervals of two to four weeks during the study.

The results showed an effect from each

of the medications despite the continuance of a usual diet. During the period of placebo administration, the average concentration of serum cholesterol was 341 mg. per 100 ml. (range 277 to 522). The average changes for the group, expressed as per cent decrease from 341, were as follows: niacin, 24 per cent; safflower oil, 6 per cent; and sitosterol, 13 per cent. There was little correlation between the response of a given patient to one agent or another. The average weight of the patients varied slightly as follows: placebo, 146 pounds; niacin, 144 pounds; safflower oil, 150 pounds; and sitosterol, 147 pounds. Safflower oil added approximately 540 calories to the daily diet.

Combined therapy of nine patients with both sitosterol and niacin (average dose 4.3 g. per day) resulted in a mean decrease in cholesterol level of 25 per cent when compared with the level during placebo administration (346 mg. per 100 ml.). On the other hand, administration of 4.3 g. of niacin alone per day to these patients caused only a 17 per cent decrease in cholesterol level, and sitosterol alone a 12 per cent decrease.

Side reactions which accompanied administration of niacin were flushing, itching and gastrointestinal distress. Occasional abnormalities were found in the results of the cephalin-cholesterol flocculation test and in the concentration of alkaline phosphatase and glutamic-oxalacetic transaminase in the serum. There was no evidence of clinical hepatic disease. There was also some impairment of the carbohydrate tolerance test. All of these abnormalities were reversed as soon as niacin was discontinued. Neither sitosterol nor safflower oil caused any discomfort other than unpalatability and a tendency to cause loose stools.

The authors discussed possible mechanisms whereby these agents might reduce the concentration of cholesterol in the serum. Presumably sitosterol inhibits absorption of cholesterol from the gut. Unsaturated oils are most effective if substituted for saturated fats, but, aside from the factor of essential fatty acids, the reason is obscure. Niacin is effective but its amide is not, even though it also is an active vitamin. The cutaneous flushing and itching seem to be inseparable from the cholesterol lowering effect. Explanations for this have ranged from an oxidative enzymatic process (J. M. Merrill, *Circulation Res.* **6,** *482 (1958)*) to enhancement of the metabolic rate (R. Altschul and A. Hoffer, *Arch. Biochem. Biophys.* **73,** *420 (1958)*). Niacin induces urinary excretion of nicotinuric acid but niacinamide does not. Whatever the mechanism, it must be a pharmacologic one since the necessary dose is so great.

This report describes an experimental method whereby hypercholesterolemia can be reduced by 25 per cent without dietary change or undue risk. This must be considered an investigational form of therapy, and should not be adopted as a routine measure until more experience has been gained. The possibility of late toxic effects needs further study. Finally, it will require critical evaluation to determine whether artificial lowering of the concentrations of cholesterol in the serum is actually of any value to the patient.

NITRATES OR NITRITES AND ABORTION IN CATTLE

Nitrates or nitrites in the diets of pregnant dairy cattle produced non-infectious abortion similar to that observed in cattle on unimproved marshland pasture. Sodium nitrite was more toxic than potassium nitrate.

The problem of non-infectious abortion in dairy cattle has concerned veterinarians and dairymen for many years. In fact, one form of this type of abortion has been reported

among cattle on unimproved marshland pasture in Wisconsin for the past 50 years. Early work from the Wisconsin Agricultural Experiment Station indicated that nitrates or nitrites present in rather high levels in certain species of weeds were probably responsible for this abortion syndrome.

J. Simon *et al.* (*J. Am. Vet. Med. Assn.* **135,** *311* (*1959*)) have recently reported on experiments in which they attempted to correlate the quantity of nitrate or nitrite in the feed with the development of an abortion in pregnant dairy heifers. Earlier observations of oat-hay poisoning (W. B. Bradley, H. F. Eppson and O. A. Beath, *Wyoming Agric. Exper. Sta. Bull.* **241** (*1940*)) indicated that the lethal dose of potassium nitrate for cattle was 25 g. per 100 pounds of body weight. E. I. Whitehead and A. L. Moxon reported (*South Dakota Agric. Exper. Sta. Bull.* **424,** *1* (*1952*)) that excessive nitrate in the diet would result in abortion.

Simon *et al.* postulated that a continuous intake of nitrate or nitrite in sufficient quantity would produce a methemoglobinemia and that the resulting anoxemia in the fetal calves would cause death and subsequent abortion. They set up experiments in which pregnant heifers were given varying levels of potassium nitrate or sodium nitrite at regular intervals in an effort to ascertain whether the resulting syndrome of abortion was identical with that occurring on the marshland pastures.

Ten grams of sodium nitrite given twice daily for three weeks produced methemoglobin levels as high as 12 per cent of the hemoglobin after one week. At the end of two weeks this had risen to 18 per cent, but did not result in abortion. One cow produced a normal calf shortly after the nitrite was discontinued, but the fetal membranes showed lesions similar to those reported for marshland abortions. Interestingly enough, 40 g. of potassium nitrate fed twice daily for three weeks gave no signs of illness or abortion and no measurable methemoglobin in the blood.

In a more critical experiment, Simon *et al.* fed potassium nitrate by capsule twice daily at levels ranging from 10 to 20 g. per 100 pounds of live weight. Those animals given 20 g. per 100 pounds of live weight died following the second dose, having received a total of 140 g. of potassium nitrate. All of the animals receiving the intermediate level, a total of 100 g. of potassium nitrate per day, aborted and the lesions of the fetal membranes resembled those seen in the naturally occurring abortions on marshland pasture. Those animals receiving 10 g. per 100 pounds of live weight did not abort, although they did develop high levels of methemoglobin in their blood.

These very interesting studies emphasize that animals on poor grass hay or pasture may be far more susceptible to nitrate or nitrite poisoning than animals on diets high in carbohydrate concentrates. Certainly this work shows conclusively that nitrates or nitrites will cause severe methemoglobinemia and that one of the results in pregnant cattle may be abortion associated with anoxemia.

It is also evident from a consideration of this work that the methemoglobin levels of blood do not consistently reflect the level of nitrate or nitrite in the diet. Furthermore there was a wide variation between different animals on the same nitrate level, suggesting that methemoglobin in the blood is a poor measure of nitrate toxicity. Apparently individual variation, diet composition, sampling technique, time from blood sampling to analysis and method of analysis all influence methemoglobin values.

Simon *et al.* suggest that some better technique of measuring the effect of nitrate and nitrite is required and propose the analysis of nitrate in the blood as a method for determining the reaction of cattle to nitrate in the feed.

While the work of these authors has established the close relationship between nitrate or nitrite in non-infectious abortion

in dairy cattle, the limited number of animals, the individual variation and the wide spread in nitrate levels used prevented the determination of levels at which this syndrome will develop. More particularly, they have not indicated the levels at which nitrite, a more toxic ingredient than nitrate, will result in abortion nor what is the likely relationship between nitrite and nitrate under naturally occurring pasture conditions. These questions are of particular importance to dairymen and cattlemen in areas where high nitrate levels in pasture forage are a problem.

BLOOD LOSS AND PLASMA CHOLESTEROL LEVELS

The removal of blood samples from rats may be responsible for increases in plasma cholesterol levels.

In many projects using small laboratory animals, the effect of diet on plasma cholesterol levels is studied by taking samples of blood at frequent intervals.

In the course of such a project, I. Coleman and J. M. R. Beveridge (*Nature* **184,** *1041 (1959)*) found that some of the increases observed in the cholesterol levels with a high-fat diet in one rat experiment were not reproduced when the experiment was repeated. The only difference in the conditions of the two experiments that might provide an explanation for this was in the frequency of the blood samples and their size.

In the first experiment, samples corresponding to 2 per cent of the blood volume of the rats were taken on alternate days for the first eight days, and then at weekly intervals for a month. In the second experiment lasting two weeks, samples corresponding to 0.5–1.0 per cent of the blood volumes were taken on the first and last day only, and there was no change in cholesterol level in the rats receiving the high-fat diet.

Two further trials were then set up to measure the response of rats to two experimental diets under different degrees of bleeding stress. In one diet, just under 60 per cent of the calories were calculated to come from corn oil, while the second diet was essentially fat-free. Each trial lasted for six days and the rats were bled on alternate days.

In the first of the two trials, using twenty animals for each diet, the initial level of plasma cholesterol was approximately 72 mg. per 100 ml. Six days later after three blood samplings (each of which were equivalent to 1.0–1.5 per cent of blood volume), the values for the high-fat diet had increased significantly by 31 per cent while those for the fat-free diet had increased by only 10 per cent (a nonsignificant difference). In the second trial, in which 15 animals were allotted to each diet, the mean initial level of plasma cholesterol was 67 mg. per 100 ml. The conditions were the same as for the first trial except that the samplings corresponded to 2.5–4.0 per cent of blood volume on each occasion. After six days, the values on the high-fat diet had increased by 122 per cent, and on the fat-free diet by 59 per cent.

With respect to experimental design, it was unfortunate that the size of blood sample was not used as a variable within a test. Nevertheless the results do strongly suggest that the taking of ordinary blood samples can itself be a factor influencing plasma cholesterol levels in experiments with rats. Work has been reported previously by J. J. Spitzer (*J. Lab. Clin. Med.* **46,** *461 (1955)*) confirming the older observations that hemorrhage could result in lipemia in rabbits, but showing that daily blood losses of the order of 10 to 15 per cent of blood volume were needed in order to observe the phenomenon. The suggestion from the present work is that very much

smaller losses can precipitate cholesterolemia in rats.

The conclusion seems to be that experimentalists should take care to equalize the blood losses of experimental animals receiving different treatments; and also that they should record in their publication what degree of bleeding stress has been imposed. As is pointed out by Coleman and Beveridge, some of the conflicting reports about the response of the plasma cholesterol level to high-fat diets may be explained by differences in bleeding frequency and sample size in different laboratories.

GROWTH RATE AND LYSINE

Evidence is offered that differences in rates of growth of chicks do not, per se, necessitate different dietary levels of lysine (expressed as a percentage of the diet) for optimum growth.

One of the explanations for the occurrence of suboptimal growth with highly purified diets apparently containing all nutrients, including amino acids, is that the rate of growth of an animal might influence its percentage requirement for essential amino acids. However, P. Griminger (*Doctoral Thesis, University of Illinois, Urbana (1955)*) found no significant differences in requirements for methionine, tryptophan and lysine between fast- and slow-growing chicks.

On the other hand, H. M. Edwards *et al.* (*Poultry Sci.* **35,** *385 (1956)*) observed that, to obtain optimum growth with diets containing wheat gluten or sesame meal, a different percentage level of lysine was required for each diet, *i.e.*, the chicks receiving the sesame meal had a higher requirement for lysine. Since these chicks also grew at a much greater rate than those receiving the wheat gluten diet, the authors interpreted this to mean that the lysine requirement of the chick is related to the rate of growth.

Because the findings of Edwards and co-workers seemed to be at variance with his own, Griminger, working with H. M. Scott (*J. Nutrition* **68,** *429 (1959)*), has carried out a number of experiments to determine whether the rate of growth actually does, per se, influence the lysine requirement (expressed as a percentage of the diet) of the growing chick. Lysine was used as the limiting amino acid since it is concentrated to the greatest degree in muscle and organ tissue. Thus it would appear likely that the requirement for this amino acid is influenced by rate of growth.

The requirement for lysine in growing chicks was determined under four different conditions: (1) with chicks from a single population but selected for fast and slow growth, (2) with and without a non-nutritive growth depressant (saponin), (3) with chicks from different breeds growing at different rates, and (4) with and without inoculation of the chicks with bronchitis virus.

In the first experiment, optimum gains, weights and growth rates for both groups were obtained at the 1.03 per cent level of lysine. However, analysis of the growth rates revealed that the terms "fast"- and "slow-growing" for the two groups were only relative. The growth results, when broken down into growth groups at two weeks of age, showed a clear tendency for the smaller chicks to add more weight on a percentage basis than the larger chicks. Consequently, there should be a smaller maintenance requirement and it was found that the gain to feed ratios were more efficient for the smaller chicks. Statistical analysis revealed that final growth data were significantly related to a particular growth group or to the lysine level, but that no significant interaction exists between these two factors.

Although administration of saponin reduced gains and growth rates to approximately two-thirds of normal, it did not alter the percentage level of lysine at which optimum growth took place. Similarly, the chicks from a highly inbred line of white Leghorns registered optimum gains and growth rates (within their genetic capabilities) at the same level of lysine (0.93 per cent). Optimum gain to feed ratios were also obtained at this level of lysine. Both the saponin and Leghorn groups had lower feed efficiency values than the control group.

In the last experiment, using the bronchitis virus as a growth depressant, optimum growth was obtained at the 1.11 per cent level of lysine for both the injected and control groups. Growth rates and gain to feed ratios followed the same pattern.

The authors conclude that the evidence from the above three experiments is overwhelmingly in favor of the concept that differences in growth rate of chicks do not, per se, necessitate different dietary levels of lysine for optimum growth. According to the authors, the findings further support H. J. Almquist's view (*J. Nutrition* **34,** *543 (1947)*) that the proportions of indispensable amino acids remain the same for any suboptimal rate of growth. Lower feed consumption would satisfy the lower absolute requirement for lysine of a slow-growing chick without changing the requirement of lysine as a percentage of the feed.

According to the authors, the lower growth rate and lower requirement found by Edwards *et al.* (*loc. cit.*) with the wheat gluten diet is probably caused by the amino acid deficiency in the test diet, without a cause and effect relationship between growth rate and lysine requirement. Thus since the low growth rate appears to result from the lack of ample amounts of balanced protein, the statement regarding the effect of a low growth rate on amino acid requirement does not appear justified.

Further strengthening the contention that rate of growth does not necessarily influence the percentage requirement for essential amino acids are the studies of H. H. Mitchell and J. R. Beadles (*J. Nutrition* **47,** *133 (1952)*), which show that the protein percentage for maximum growth of rats was independent of caloric intake level when the caloric density of the diet remained the same. D. E. Becker *et al.* (*J. Animal Sci.* **13,** *346 (1954)*) found that a moderately depressed feed intake of pigs did not influence the protein requirement, and H. D. Hutchinson (*Doctoral Thesis, Univ. of Illinois (1957)*) observed that, when lysine was the limiting factor in the basal ration, neither the level of feed intake nor the consequent difference in growth rate influenced the lysine requirement of the weanling rat.

Other authors have offered evidence suggesting that different breeds may have differing amino acid requirements. D. M. Hegsted *et al.* (*J. Biol. Chem.* **140,** *191 (1941)*) concluded that higher arginine and glycine levels were required for fast-feathering breeds of chicks during times of feather development. M. W. McDonald (*Australian J. Agr. Res.* **9,** *161 (1958)*) attributed his findings on Australorps to a specific metabolic block in sulfur amino acid metabolism rather than to a difference in growth rate between breeds.

COLD AND RIBOFLAVIN REQUIREMENT

The requirements for riboflavin are proportional to intake of food even under exposure to cold.

The concept that the requirements for riboflavin and other B vitamins are proportional to intake of food has been challenged by H. H. Mitchell *et al.* (*J. Nutrition* **41,**

317 (1950)) and by B. H. Ershoff (*Proc. Soc. Exp. Biol. Med.* **79,** *559 (1952)*) who subjected their experimental animals to low temperatures. Riboflavin is known to be involved in the production of the adrenocortical hormones, which are in turn concerned with the physiological response to cold. Moreover, riboflavin plays a coenzymatic role in the catabolism of fat, the importance of which may be increased by the presence of cold.

However, despite this evidence and reasoning, O. A. Bessey *et al.* (*J. Nutrition* **64,** *185 (1958)*) found that there was no increase in riboflavin utilization by riboflavin-deficient rats when metabolism was increased by exposure to cold. M. Fontaine and A. Raffy (*C. R. Soc. Biol.* **136,** *297 (1942)*) are quoted by these workers as observing that rats exposed to cold had a 20 per cent higher concentration of riboflavin in their livers.

D. A. Vaughan and L. N. Vaughan (*J. Nutrition* **68,** *485 (1959)*) applied a more critical test to the idea that the requirement of rats for riboflavin might be dependent on factors other than food intake. They reasoned that if food intake is the most critical determinant of riboflavin needs, animals subsisting in the cold, when allowed to fill their cold-induced energy requirements, should be as well off as their controls at normal temperature when eating the same levels of riboflavin per unit of food.

To test this hypothesis, they used two groups of 25 male Sprague-Dawley rats ranging in weight from 175 to 250 g. One group was placed in individual cages in a cold room held at 5 ± 2° C. The other group was maintained at 25° C. All rats were placed on a riboflavin-deficient diet until growth ceased or fluctuated about a central point. The deficient diet consisted of: vitamin test casein (18 per cent), sucrose (68 per cent), vegetable oil (10 per cent), and U.S.P. Salt Mixture No. 2 (4 per cent). The vitamin mixture supplied: 2000 units of vitamin A, 222 units of vitamin D, 11.1 mg. of alpha-tocopherol, 100 mg. of ascorbic acid, 11.1 mg. of inositol, 166.5 mg. of choline chloride, 5 mg. of menadione, 11.1 mg. of para-aminobenzoic acid, 10 mg. of niacin, 22.2 mg. of pyridoxine hydrochloride, 6.66 mg. of calcium pantothenate, 44 micrograms of biotin, 200 micrograms of folic acid, 3 micrograms of vitamin B_{12}, and 2.22 mg. of thiamine hydrochloride per 100 g. of diet.

At the end of 28 days, growth had ceased in both groups of animals, at which time each group of 25 rats was divided into five sub-groups receiving 0.5, 1.0, 1.5, 2.0 or 4.0 micrograms of riboflavin per gram of diet (4.0 micrograms per gram was assumed adequate for maximum growth).

It was found that the growth response of rats receiving the same level of riboflavin in the diet was unaffected by the environmental temperature when the riboflavin level was 2.0 micrograms or less. At the 4.0 micrograms per gram level, however, the growth of the rats kept at normal temperature was significantly greater than that of corresponding rats maintained in the cold.

These results do not agree completely with those of Mitchell *et al.* (*loc. cit.*) who observed that in growing pigs the riboflavin requirement per unit of food was greater at 5° than at 29° C. Ershoff (*loc. cit.*) also reported impaired survival and depressed growth in rats subjected to cold. Vaughan and Vaughan explain, however, that in Ershoff's experiments the animals were already deficient when placed in the cold room, while their animals were allowed to become deficient at 5° C, a procedure permitting them to become adjusted to cold before being subjected to the additional stress of the deficiency.

Both the level of riboflavin in the food and exposure to cold had a significant effect on food intake, but there was no significant interaction between these conditions. Food intake rose rapidly with increasing levels of riboflavin until the 1.5 microgram level was reached, whereupon the increment became

quite small. The differences in food intake between normal and cold rats (cold rats ate more) were quite uniform across successive levels of riboflavin. Apparently the portion of the appetite stimulated by the exposure to cold was not affected by the anorexia induced by the deficiency in riboflavin.

When the final weights of the rats were adjusted for food intake and initial weight, it was obvious that the rats held at 25° C used their food more efficiently for growth. In addition, multivariate analysis showed that the level of riboflavin had a significant effect on the adjusted final weights, indicating that the efficiency of food utilization for growth, as well as food intake, was lowered in riboflavin-deficient rats at both temperatures.

PHYSIOLOGICAL PROPERTIES OF DITHIOPROPYLTHIAMINE

Dithiopropylthiamine produces higher blood and urine thiamine and higher liver cocarboxylase than does thiamine itself by virtue of its greater absorption rate.

Allithiamine is the derivative formed by the reaction of allicin (diallyl disulfide, monosulfoxide) in the gut or the test tube, with the ring sulfur of thiamine. The ring is thus opened and the allyl thiol radical becomes attached to thiamine in a disulfide linkage. Allicin itself is formed from alliin (s-allylhomocysteine sulfoxide) by the enzyme alliinase, both enzyme and substrate occurring in the garlic (allium sativum) and certain related species. M. Fujiwara and his co-workers found, in a series of experiments, that allithiamine is more rapidly absorbed from the intestine than is thiamine itself and thus gives higher blood levels of thiamine with greater rapidity (apparently, allithiamine undergoes ring closure to thiamine in the body) (*Nutrition Reviews* **16,** *336 (1958)*).

In a further attempt to study the physiological properties of compounds of this type, D. Hioco, R. Tixier and A. Uzan (*Bull. Soc. Chim. Biol.* **41,** *1075 (1959)*) undertook experiments on animals and man with dithiopropylthiamine, a compound with the same structure as allithiamine but with the propyl replacing the allyl group.

Three types of animal experiments were carried out. In the first, doses of 100 to 10,000 micrograms of either dithiopropylthiamine or thiamine hydrochloride were given orally to rats, and their liver cocarboxylase (thiamine pyrophosphate) was measured after one hour. Control animals, which had received no supplement, had an average of 6 micrograms of cocarboxylase per gram of liver. The highest value obtained using thiamine itself was about 10, while that for dithiopropylthiamine was 20 micrograms per gram. In the second experiment, 250 micrograms of both compounds were given orally to the rats and the cocarboxylase was measured at one, two, four and six hours. The liver cocarboxylase rose more rapidly with the dithiopropylthiamine and to about twice the level obtained with thiamine itself. In the third experiment, the duration of the effect was studied by measuring liver cocarboxylase six hours after ingestion of either thiamine or dithiopropylthiamine. As before, the levels following dithiopropylthiamine were about twice those following thiamine regardless of dose size.

In the human experiments, a 100 mg. of dithiopropylthiamine or thiamine hydrochloride were given orally to four or five subjects and the blood levels of thiamine were measured at one and one-half and four hours. Little rise above normal was found after thiamine ingestion whereas, after ingestion of dithiopropylthiamine, elevation to between 15 and 100 micrograms per 100 ml. of blood occurred at 90 minutes and levels between 10 and 30 micrograms were observed even four hours after dosage. Moreover, urine levels, which are a reflection of

intestinal absorption rate, were several times higher with dithiopropylthiamine than with thiamine.

It has thus been shown that this derivative of thiamine, by virtue of its rapid intestinal absorption, causes far more rapid attainment of high blood levels of thiamine and of liver cocarboxylase than does thiamine itself. Its low toxicity, when compared with thiamine itself, also recommends it.

VITAMIN SPARING BY POORLY DIGESTED CARBOHYDRATES

Poorly digested sugars promote proliferation of intestinal bacteria which synthesize sufficient B-vitamins for the needs of mammals.

It has been shown by T. B. Morgan and J. Yudkin (*Nature* **180,** *543 (1957)*; *Nutrition Reviews* **16,** *126 (1958)*) that rats on a diet devoid of the B-vitamins can thrive if the diet contains 20 per cent sorbitol. In order to study this phenomenon further, H. Haenel, H. Ruttloff and H. Ackermann (*Biochem. Z.* **331,** *209 (1959)*) have carried out experiments designed to confirm and amplify the original observations.

Four groups of weanling rats were placed on the following diets: (1) a basic diet consisting of 55 per cent sucrose, 15 per cent cocoa butter, 20 per cent casein, 5 per cent cellulose, 5 per cent salt mixture and adequate proportions of the vitamins mixed daily with the diet; (2) the basic diet without the B-vitamins; (3) the basic diet without the B-vitamins but with 20 per cent sorbitol replacing a like amount of sucrose; (4) a natural diet (grains, milk powder, etc.).

The animals on the natural diet gained the most weight (to 180 g. at 60 days), followed by the vitamin-supplemented group (to 150 g.). The vitamin-deficient group lost weight and all had died within 45 days. Inclusion of 20 per cent sorbitol in this diet, however, prevented obvious vitamin deficiency diseases and permitted some weight gain (to 135 g.). These results confirmed the previous report of Morgan and Yudkin (*loc. cit.*) in most respects. However, the sorbitol-fed animals did not grow quite normally and responded irregularly to the treatment in that a few gained no weight and died with symptoms of vitamin deficiency. Moreover, addition of sorbitol to the diet of the deficient animals brought about slow recoveries as compared with a B-vitamin supplement.

Microbiological analyses of the urines confirmed the beneficial effect of the sorbitol. Urine contents (reflecting blood levels) of thiamine, niacin, riboflavin and vitamin B_6 were lowest for the vitamin-deficient group, intermediate for the sorbitol group and highest for the vitamin-supplemented group. The implication was thus that sorbitol, in some way, was effecting higher concentrations of these vitamins in the intestines and, hence, in the blood of the deficient animals.

Analysis of 24-hour urine samples of all groups of rats revealed that of those on the artificial diets, the sorbitol-supplemented group had the highest concentrations of indican (indoxyl sulfate, a product of bacterial alteration of indole, indicative of greater bacterial action). The group on the natural diet, however, had somewhat higher indican concentrations.

Further experiments showed that lactose, also, had some vitamin-sparing action, although it was only about half that of sorbitol. Investigation of the rate of absorption of these two carbohydrates revealed that they persisted throughout the small intestine, whereas the readily absorbable sugars (glucose, sucrose and fructose) disappeared in the top portion of the duodenum.

These results led the authors to what appeared to be the obvious conclusion; that those carbohydrates that are absorbed with difficulty promote the proliferation of intestinal bacteria which synthesize the B-

vitamins for absorption by the host animal.

This conclusion is also shared by L. A. Cherkes and A. A. Dinerman (*Biokhimiya* **24,** *329* (*1959*)), who studied the effect of dietary carbohydrate on development of choline deficiency in rats. On the basic choline-deficient diet (consisting of peanut-cake meal, 30 per cent; casein, 6 per cent; lard, 18 per cent; sucrose, 40 per cent; gelatin, 3 per cent; salt mixture, 4 per cent and adequate amounts of B-vitamins) weanling rats developed severe hemorrhagic degeneration of the kidneys, one of the typical symptoms of this state (W. H. Griffith, *Biological Action of the Vitamins, p. 169. Univ. of Chicago Press (1942)*).

Substitution of 20 per cent sorbitol and 20 per cent lard for the sucrose in a second group of rats resulted in almost complete elimination of the kidney damage. The diarrhea accompanying this diet, however, was so severe that a third group of rats was gradually adapted to the sorbitol diet by stepwise replacement of the sucrose by sorbitol. In this group, three of 11 animals showed moderate to severe kidney damage.

Examination of the three groups of rats for fatty infiltration of the livers revealed a somewhat different picture. The first group, as was expected, showed severe fatty infiltration. The second group had considerably lower liver fat, but it was thought that this might be from the effect of the diarrhea, which lowered the caloric intake and reduced the weight gain. This is highly probable since the third group, which did not have diarrhea and showed normal weight gains, had liver fat contents about as high as those of the first group.

The authors concluded that the amount of choline furnished by the influence of the sorbitol, while sufficient to prevent kidney damage, fell short of the requirement needed to prevent fatty livers. They should also have considered that their sorbitol-containing diets were very high in lard as compared to the sucrose diet and that this factor alone might have influenced the development of fatty livers.

Whether this knowledge can be put to practical use in human nutrition has not been determined, but it undoubtedly represents an outstanding example of the importance of intestinal bacteria in the nutrition of mammals, and should be considered in all such experiments.

LIME-TREATED CORN

Alkali or lime treatment converts the bound niacin in corn, unavailable to animals, to a free available form, probably explaining the absence of pellagra in countries where tortillas are eaten in quantity.

Although corn is the primary staple in those parts of the world where pellagra is endemic, it has been recognized for a number of years that corn contains considerable amounts of niacin. It was observed that only a part of the niacin could be extracted from corn with water, which led to the suggestion that niacin existed in a free and a bound form. The latter was believed unavailable to man and animals (*Nutrition Reviews* **15,** *53* (*1957*)).

The absence of pellagra in such corn-eating countries as those in Central America was attributed to the liberation of the bound niacin during the preparation of the corn for domestic use. The lime treatment of the corn, used universally in that part of the world, presumably converted the bound niacin to the free form, thus increasing the amount of niacin available to the consumer.

A number of studies have been made to assess the efficacy of the lime treatment in converting corn to an anti-pellagragenic food. One of these was the study by G. A. Goldsmith and co-workers (*Nutrition Reviews* (*loc. cit.*). These investigators, however,

failed to find any difference respecting pellagra-curing activity between corn made into tortillas, as in Central America, and corn prepared according to the methods used in the southern part of the United States when that area had a high incidence of pellagra. The investigators pointed out that the primary limitation of their study was the small amount of corn in the diet fed the subjects (15 to 20 per cent of the calories). In Central America tortillas are a major constituent of the diet and it is possible that the large amount of lime-treated corn used in that part of the world may increase the intake of free niacin to such an extent that it prevents pellagra.

For many years, E. Kodicek has studied the availability of niacin in different foods. As early as 1940 (*Biochem. J.* **34,** *712 (1940)*), when he was studying the conditions that influence the cyanogen bromide procedure, he found that in such animal tissues as liver and muscle all of the niacin could be extracted with boiling water. However, only a small fraction of the material in corn which gave the color test for niacin could be extracted with water. On the other hand, hydrolysis with dilute alkali extracted so much niacin from corn that, if it were all true or active niacin, "yellow maize which is known to be deficient would have a high concentration of nicotinic acid."

Physiological verification that only a small part of the niacin in corn is active or free came from Kodicek's comparison of the rate of production of blacktongue in dogs receiving either a corn or a purified diet. Under these circumstances the corn diets, which contained from 12 to 27 micrograms of niacin per gram by chemical analysis after alkaline hydrolysis, had the same effect as a purified diet containing only 7 micrograms of niacin per gram.

On the basis of work such as this, many studies were carried out with animals fed corn which had been soaked in dilute sodium hydroxide or lime water. These animals did not develop symptoms of a niacin deficiency, whereas the controls fed the untreated corn did (Kodicek, R. Braude, S. K. Kon and K. G. Mitchell, *Brit. J. Nutrition* **13,** *363 (1959)*).

Since most of the preceding work had been done with rats and chicks, Kodicek, Braude, Kon and Mitchell (*Brit. J. Nutrition* **10,** *51 (1956)*) tested the alkali-hydrolyzed corn as a curative agent for niacin-deficient pigs. In so far as niacin requirements are concerned, the pig more closely resembles man than does the rat. Analysis of the milling fractions indicated that 73 per cent of the total niacin in corn occurred in the hominy meal and bran, which represented 27 per cent of the corn. In view of this fact, 265 pounds of the mixture of hominy meal and bran were treated with 400 liters of 0.5 N-sodium hydroxide at a temperature of 60°. The alkali was neutralized with 10 liters of concentrated hydrochloric acid and then the mixture was dried in a heating tunnel at 70°. The dried, hydrolyzed mixture was mixed with the remainder of the corn and incorporated at a level of 78 per cent in a diet containing 10 per cent pea meal, 5 per cent casein, 3 per cent cod liver oil, and 2.5 per cent salts.

When pigs weighing 37 to 59 pounds were maintained on a diet similar to the above except that untreated corn was used in place of the alkali-treated corn, the animals stopped growing and showed symptoms of a niacin deficiency in about 35 days. At this time, six pigs were supplemented with 6 mg. of niacin per day. Another six were changed to the diet containing the alkali-treated corn. Both groups of pigs immediately began to gain weight, with the niacin-supplemented ones gaining 5.0 ± 0.33 pounds per week and those fed the alkali-treated corn gaining 3.2 ± 0.62 pounds.

Chemical analysis and paper chromatography confirmed the fact that all of the niacin in the untreated corn was in the bound form, whereas all in the alkali-treated corn was in the free form (Kodicek *et al.*, *loc. cit.*).

To round out the above studies, Kodicek

and co-workers (*Brit. J. Nutrition* **13,** *363 (1959)*) repeated their work with corn treated with lime water according to the method used in Mexico for preparing tortillas. One ton of corn was so treated and then baked in the form of flat cakes to make tortillas. Another three tons of corn from the same batch were used in the diets fed to the pigs to produce the deficiency state.

Chemical analysis indicated that the untreated corn contained 17.7 micrograms per gram of niacin, but that only 0.3 micrograms of this was in the free form. The tortillas, on the other hand, contained 11.0 micrograms of niacin per gram of dry material, all of which was in the free form. The lime treatment resulted in a reduction of the thiamine content of the corn from an original value of 4.6 micrograms per gram to 0.39. The riboflavin was reduced from 0.43 micrograms per gram to 0.27, calcium pantothenate from 3.4 to 1.1 micrograms, vitamin B_6 from 3.4 to 1.7 micrograms, folic acid from 70 to 8 millimicrograms, and the tocopherols from 16.0 to 4.6 micrograms. Synthetic vitamins were added to the diet containing tortillas to compensate for these losses.

Eighteen weanling pigs were maintained on the untreated corn diet until they ceased to gain or lost weight for seven days. Six pigs were then maintained on the diet as controls. Two of these animals died showing typical signs of a niacin deficiency, while the other four gained weight at the rate of 1.7 ± 1.43 pounds per week. Another six pigs received 6 mg. of niacin per day, which was in addition to the 27.5 mg. each one received from the diet. Of the latter, only 3.4 mg. was in the free form, making a total intake of 4.4 mg. of free niacin per day. These animals gained 6.5 ± 0.42 pounds per week. The six pigs on the tortilla diet received 19.9 mg. of niacin per day, all of which was in the free form, and these animals gained 7.9 ± 1.15 pounds per week.

There was a correlation coefficient of + 0.86 between the intake of free niacin and the gain in body weight; the coefficient for total niacin was + 0.60. Although the two correlation coefficients did not differ significantly, the higher correlation coefficient for the free niacin suggested to the investigators that the substance in the tortillas responsible for the body weight gains was the free niacin.

At the end of the experiment, the animals were killed and the livers assayed for niacin. Here again there was a correlation coefficient of + 0.81 between the intake of free niacin and the content of this vitamin in the liver. In this case, the correlation coefficient for the total niacin was + 0.30. These two correlation coefficients differed significantly.

The investigators concluded that the beneficial effect of tortillas in curing niacin deficiency in pigs could be attributed entirely to the conversion of bound niacin in the corn to the free form as a result of the lime treatment. They present considerable evidence to substantiate their conclusion that changes in availability of proteins or of amino acids could not explain the difference between the corn before and after lime treatment.

ENZYME CHANGES IN GALACTOSE CATARACT

Both in vivo and possibly in vitro galactose-1-phosphate inhibits the activity of glucose-6-phosphate dehydrogenase activity in rat lenses.

One of the experimental methods of producing cataracts in the eyes of animals, a condition where the lens becomes opaque or clouded, is to maintain them on diets with a high content of galactose. Moreover, infants with a hereditary disorder termed galactosemia may develop cataracts of the eye (*Nutrition Reviews* **14,** *5 (1956)*). H. M. Kalckar and associates (*Science* **125,** *105, 113 (1957)*) noted that the liver and red

blood cells of patients with galactosemia have a relative and absolute decrease in the activity of the enzyme galactose-1-phosphate uridyl transferase. This enzyme converts galactose-1-phosphate to a glucose-1-phosphate. It has also been noted by V. Schwarz and L. Golberg (*Biochim. et Biophys. Acta.* **18,** *310 (1955)*) that there is an accumulation of galactose-1-phosphate in the lens capsule and epithelium of rats maintained on a diet containing 30 per cent galactose. There was about ten times the concentration of galactose-1-phosphate in the lenses of the rats fed galactose as compared to the lenses of normal rats. In the metabolism of carbohydrate by the lens, J. H. Kinoshita (A.M.A. Arch. Ophth. **54,** *360 (1955)*) reported that the hexose monophosphate shunt is of considerable importance.

Recently, S. Lerman (*Science* **130,** *1473 (1959)*) studied the possibility that galactose-1-phosphate might directly inhibit some steps in the metabolism of glucose. To do this he assayed the levels of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the lenses of normal rats and those fed high levels of galactose. Twelve animals were in each of the two groups. When the first signs of cataract formation were detected by ophthalmoscopic examination (peripheral subcapsular vacuolization) in the animals fed the galactose, both groups were sacrificed and the content of these dehydrogenases was determined in the lenses. This defect usually occurred on the twelfth to fourteenth day after beginning the galactose feeding.

The level of the glucose-6-phosphate dehydrogenase in the lenses of the control animals averaged 25.2 units (23.7 to 26.9), while the level of this enzyme in the lenses of the animals fed the galactose averaged 13.8 (7.9 to 18.8) units. Thus there was a decrease of approximately 45 per cent in the activity of glucose 6-phosphate dehydrogenase in the lenses of the animals consuming galactose. No differences were noted in the enzyme activity of 6-phosphogluconate dehydrogenase between the animals fed galactose (6.25 units) and those receiving glucose (6.16 units).

In another series of studies, Lerman maintained two groups of 14 animals each on the diets described above and sacrificed one pair of control (glucose-fed) and one pair of test animals (galactose-fed) on the fourth, sixth, seventh and eighth day of the feeding schedule. The remaining pairs of animals were sacrificed on the sixteenth, ninteenth and twentieth day of the study. Cataracts were present by the end of the study. The lenses of each animal were assayed for both glucose-6-phosphate and 6-phosphogluconate dehydrogenases.

There was a progressive fall in the level of glucose 6-phosphate dehydrogenase activity in the lenses of the animals receiving the galactose diets. The difference was noted by the fourth day of feeding and by the fourteenth day the activity fell to about 60 per cent of normal, remaining at this decreased level until the end of the study. No difference was noted between the levels of 6-phosphogluconate dehydrogenase in the two groups of animals. Thus it appeared that there was an inhibition of the glucose-6-phosphate dehydrogenase activity *in vivo* when the animals were fed the galactose.

Lerman then undertook studies to determine whether an inhibition could be produced *in vitro*. Lenses from five normal rats were studied. One pair of lenses was homogenized in 2 ml. of distilled water and the insoluble protein removed by centrifugation. Half of each portion of the soluble protein fraction was incubated in a media containing an excessive amount of galactose-1-phosphate compared with glucose-6-phosphate. The remaining portion of the lens homogenate was incubated with glucose-1-phosphate as substrate. After three hours of incubation at 37° C an aliquot was assayed for the combined glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activity. By comparing the two

groups, it was found that there was an average inhibition of 21 per cent (16 to 35 per cent) when an excess of galactose-1-phosphate was present in the media. It should be noted, however, that only five animals were used in this preliminary study and the range of inhibition was broad.

Since all these aliquots contained some glucose-6-phosphate, another study was set up with the soluble homogenate derived from four sets of normal rat lenses, one half of which was incubated with 15 micromoles of galactose-1-phosphate and the other half with a similar amount of sucrose. There was an average inhibition of 20 per cent of the activity of the glucose-6-phosphate dehydrogenase while the 6-phosphogluconate dehydrogenase was not inhibited.

This investigator noted that galactose-1-phosphate is capable of inhibiting specifically glucose-6-phosphate dehydrogenase *in vivo* and may also do it *in vitro*. Thus the inhibition of the hexose monophosphate shunt is implicated in the pathogenesis of experimental galactose cataracts in rats. A similar mechanism may also be involved in the cataracts occurring in infants with galactosemia.

GASTRIC HYPERTROPHY IN FASTED RATS

Adaptation to intermittent starvation permitted 132 g. rats to consume 56 g. of feed during one night following three days of fasting. After 17 weeks of such a regimen the stomach was markedly increased in size with hypertrophy of the walls.

When individuals who are accustomed to eating a diet containing a fairly large percentage of fat are given one that is low in fat, a number of days ensue before they are able to adequately increase their food intake. During this period of adaptation there occurs an increase in the capacity of the stomach, after which time the individual is able to consume a volume of food which ordinarily would have caused him a great deal of distress.

A somewhat analogous adaptation has been studied in rats by E. Holeckova and P. Fabry (*Brit. J. Nutrition* **13**, *260 (1959)*). These investigators subjected 100 to 200 g. female rats to increasing periods of starvation. The fasts, however, were interspersed by single days when the animals had free access to a mixed diet containing 53 per cent of the calories from carbohydrate, 25 per cent from protein and 22 per cent from fat. When 24 rats were starved every alternate day, their body weight increased slightly during the first week but then plateaued for the remaining three weeks. When these same rats were fed only three days each week, their body weight again increased slightly, but plateaued during the third week, remaining constant for the next four weeks. However, when these rats were fed only twice a week, there was a gradual loss of body weight which leveled out at the original weight before starvation.

The control rats that were fed the diet on an ad libitum basis showed a variation in daily food intake ranging from 15 to 20 g. per rat per day. When the weekly food consumption was computed on a per diem basis for the intermittently starved rats, there was a progressive increase throughout the experimental period. The values for the latter group increased from approximately 10 g. per rat per day at the start of this period to 15 g. at the end.

As a result of intermittent feeding, the rats showed a tremendous increase in their capacity to consume food whenever it was available. The amount of feed each rat consumed on the day it was fed increased from 16 g. at the start of the study to 50 g. at the end. This adaptation permitted the intermittently starved rats to consume 112

g. of food in only two days during the last week of the study, while the non-starved rats ate 133 g. in the seven days.

The intermittently starved rats not only consumed more food during a given time than the controls, but retained food in their stomachs for a longer period of time. During the night following three days of starvation, the rats consumed 56 g. of food. X-rays of these 130 g. rats revealed that the stomach occupied the greater part of the abdomen. Twenty-four hours later, during which no food had been available to them, their stomachs still contained much more food than the controls that had been eating 20 g. of food per day.

The average weight of the stomachs in the 18 rats that had been intermittently starved for six weeks was 988 ± 42 (S.E.) mg. The body weight of these rats averaged 132 ± 4.9 g. The average weight of the stomachs in the 18 control rats was 937 ± 30 mg.; the average body weight was 177 ± 4.4 g. After 17 weeks of intermittent starvation, the average stomach weight for the 24 rats was 1450 ± 62 mg. for the rats weighing on an average 173 ± 5.2 g. In the controls, the average stomach weight was 1146 ± 33 mg., while the body weight was 234 ± 6.6 g. The major increase in the size of the stomach occurred in the "translucent" part (presumably the cardiac portion). The increase in the "opaque" part (presumably the pyloric portion) of the stomach was relatively small. Histological examination showed hypertrophy of all parts of the stomach, but it was most pronounced in the translucent part.

Holeckova and Fabry mentioned in their discussion additional publications not available to the reviewer. Therein they reported that the weight of the small intestine was also increased in intermittently starved rats. Associated therewith was an increased absorption of glucose and an increased alkaline phosphatase activity. The hypertrophy of the stomach and small intestine and the increased enzyme activity occurred in spite of the fact that other organs of the animal and the body as a whole lost weight.

This is an interesting and unique study on the effect of adaptation in the animal to periods of starvation. It suggests that additional studies and observations should be made of similar conditions in man, especially in those individuals subjected to marked changes in dietary practices. These changes are likely to appear in individuals who heed the advice of food faddists recommending such diets as those very low in fat.

ASCORBIC ACID IN THE PARENTAL DIET AND CALCIUM UTILIZATION BY THE CHICK

Factors other than vitamin D may sustain normal intestinal absorption of calcium in the chick.

The presence of ascorbic acid in the maternal diet influences the gastrointestinal absorption of calcium by chicks, whereas vitamin D is not involved primarily with intestinal absorption of calcium but rather is essential for normal bone development. These conclusions by P. A. Thornton, C. W. Weber and R. E. Moreng (*J. Nutrition* **69,** *33 (1959)*) are based on studies arising out of unusual observations made during the investigation of rates of feed movement in the intestinal tract and of blood concentrations and tibial uptake of Ca^{45} in control and rachitic chicks (the progeny of parents fed a ration containing ascorbic acid, a nutrient synthesized by the chicken and hence regarded as a nonessential dietary constituent).

Previous investigations (R. Nicolaysen and N. Eeg-Larsen, *Vitamins and Hormones* **11,** *29 (1953);* G. H. Bourne, *The Biochemistry and Physiology of Bone, p. 561. The*

Academic Press, Inc., New York (1956); J. M. Leichsenring, L. M. Norris and M. L. Halbert, *J. Nutrition* **63,** *417 (1957)*) have considered ascorbic acid and vitamin D separately with respect to calcium absorption and metabolism. The present authors, on the other hand, have begun to investigate the influences of ascorbic acid and vitamin D on calcium absorption and metabolism by studying the two vitamins simultaneously.

The works reviewed by Nicolaysen and Eeg-Larsen (*loc. cit.*) have shown experimentally that vitamin D is necessary for the intestinal absorption of calcium or phosphorus or both and, in addition, for normal bone deposition. In other words, it has been suggested that vitamin D functions in promoting normal bone development either by increasing calcium and phosphorus absorption or by promoting calcium and phosphorus metabolism after absorption. The studies reviewed by Bourne (*loc. cit.*) have reported both an increase and no increase in retention of calcium by rats fed orange juice and ascorbic acid and have suggested that calcium may be absorbed from the intestine as calcium ascorbate. Leichsenring, Norris and Halbert (*loc. cit.*) have shown that both orange juice and crystalline ascorbic acid increase calcium retention in women.

In the present experiments, Thornton, Weber and Moreng used New Hampshire-Delaware cross chicks which were fed ground milo until three days of age when they were divided in a random manner, group one being given a vitamin D_3-deficient ration, and group two the same ration supplemented by 13,500 I.C.U. of vitamin D_3 per 100 pounds of ration. The deficient ration contained the respective percentages of the following ingredients: ground milo, 70; soy bean oil meal, 25; ground alfalfa meal, 1.50; ground limestone, 0.50; steamed bone meal, 2.50; iodinized salt, 0.50; manganese sulfate supplement, 0.022; and vitamin mix, 0.113. In about three weeks, the deficient ration produced the rachitic symptoms of an ungainly walk, softness of the beak, unthrifty appearance and slow growth. Ten chicks were used in each control and experimental group with an equal distribution when possible between males and females.

At four weeks of age, the chicks received directly into the lumen of the crop injections of 0.5 ml. of a solution containing approximately 10 microcuries of Ca^{45} (in the form of $CaCl_2$ in hydrochloric acid solution) plus 15 mg. of Evans Blue dye. The birds were sacrificed by ether-induced asphyxiation at 15, 30, 45 and 60 minutes following injection. Just prior to sacrifice, a sample of heart blood was collected in a tube containing an 0.2 per cent solution of sodium oxalate. After sacrifice the non-calcium-absorbing "upper bowel" (esophagus, crop, proventriculus and ventriculus) were removed as a unit and prepared for radioassay. The tibiae were removed and measurements made of the epiphyseal-cartilaginous plates. The "upper bowels" and tibiae were ashed at 700° C, the ash taken up in 25 per cent HNO_3 solution, brought to a constant volume and an aliquot assayed for Ca^{45} activity in a gas flow, windowless type scaler. Statistical differences between groups were determined by calculating the standard error and applying the "t" test to such data.

The data acquired in these studies were different from those reported previously by B. B. Migicovsky and J. W. S. Jamieson (*Canad. J. Biochem. Physiol.* **33,** *202 (1955)*) and by K. W. Keane, R. A. Collins and M. B. Gillis (*Poultry Sci.* **35,** *1216 (1956)*). These authors previously reported that the specific activity of Ca^{45} in the blood reached a maximum in control chicks ten minutes after injection, declining progressively thereafter, while specific activity in the blood of deficient chicks reached a maximal activity in 30 to 90 minutes but was at a much lower level than in the controls.

Thornton, Weber and Moreng, on the other hand, observed that Ca^{45} blood levels, expressed as per cent of Ca^{45} recovered, had

risen sharply in both control and deficient chicks by 15 minutes after injection. They explain this by the rapid, exponential rate of feed movement from the "upper bowel" to the absorptive "lower bowel". At 30 and 45 minutes, Ca^{45} blood levels were similar to those at 15 minutes, but at 60 minutes Ca^{45} blood levels in both groups were again increased. The amount of Ca^{45} deposited in the tibiae were markedly different between control and deficient groups at 60 minutes. These differences were directly correlated with the amount of Ca^{45} leaving the upper bowel, the deficient group failing to send enough calcium from the "upper" to the absorptive "lower" bowel.

In seeking explanations for the differences, the authors observed that their experiments resembled those of Migicovsky and Jamieson (*loc. cit.*) and Keane, Collins and Gillis (*loc. cit.*) except that ascorbic acid had been added to the parental diet in the authors' experiments. The correlation between the amount of Ca^{45} leaving the "upper bowel" and that recovered from the tibiae in chicks from parents not receiving ascorbic acid was not significant for the deficient chicks but was highly significant for the control groups, whereas the correlation in chicks from parents receiving ascorbic acid was highly significant in both control and deficient chicks.

These observations led Thornton, Weber and Moreng to set up a "pilot study" to observe chicks from hens receiving 0, 10, 20, or 40 mg. of ascorbic acid per pound of ration. The tibial uptake of Ca^{45} was increased (to a highly significant degree, statistically) in vitamin D_3-deficient females when the parents received ascorbic acid. Epiphyseal plate widths were wider in both females and males. There was a good correlation between epiphyseal plate widths, tibeal ash weights and the amount of Ca^{45} taken up by the tibiae in the chicks from unsupplemented parents but none in chicks from supplemented parents.

The presence of ascorbic acid in the parents' diet seemed, in the authors' opinion, to have increased the severity of rickets despite the chicks' ability to deposit more calcium in bone.

In view of their data, original and derived, the authors offer four suggestions: (1) calcium absorption per se will not necessarily prevent rachitic symptoms; (2) the primary function of vitamin D_3 is not necessarily the promotion of intestinal absorption of calcium; (3) ascorbic acid or some agent produced by the presence of this vitamin in the parental diet helps maintain calcium in rachitic progeny; and (4) the presence of of this vitamin in the parents' diet may increase the degree of rickets in progeny given vitamin D_3-deficient diets.

The authors' investigations of the role of ascorbic acid in the parental diet seem to have arisen incidentally and hence for the moment have a flavor of retrospective research. Nonetheless, they are provocative and challenge some fundamental concepts of calcium metabolism. Further investigations extending their pilot study of chicks and, hopefully, of other species including the guinea pig will be anticipated. Opportunities for comparable studies in man when suitable clinical situations are presented should be anticipated.

NOTES

Letters to the Editor

Dear Sir:

We have read the article "Vitamin K Deficiency in Newborn Infants" (*Nutrition Reviews* **17,** *229 (1959)*) and should like to call your attention to the statement therein in which our paper "The Relation of Vitamin K Deficiency to Hemorrhagic Disease in the Newborn" (H. Dam, H. Dyggve, H. Larsen and P. Plum, *Advances in Pediatrics* **5,** *129 (1952)*) is mentioned as supporting the idea that prophylactic vitamin K administration to mothers or infants is of doubtful value in the newborn period.

This quotation is not in accordance with the content of our paper, and we do not understand how our paper could have led authorities to state that the value of routine prophylactic vitamin K administration was not clearly established (*Recommended Dietary Allowances, National Academy of Sciences-National Research Council Publication 589 (1958)*), as mentioned in the article in *Nutrition Reviews*.

Our studies, described in *Advances in Pediatrics* **5,** *129 (1952)*), demonstrate the efficacy of antenatal vitamin K prophylaxis against decrease of prothrombin activity and several forms of hemorrhage in the infant in the period immediately after birth.

Henrik Dam
Holger V. Dyggve
E. Hjalmar Larsen
Preben Plum
Copenhagen, Denmark

Editor's Note: It was not the work of H. Dam *et al.* but the work of Edith L. Potter (*Am. J. Obstet. Gynec.* **50,** *235 (1945)*) which led some authorities to state that the value of routine prophylactic vitamin K administration was not clearly established.

Dear Sir:

Concerning the letter of H. Cremer and D. Hotzel (*Nutrition Reviews* **18,** *63 (1960)*), I would like to point out that the stimulatory effect of penicillin on thiamine biosynthesis in the intestinal tract has been reported (*J. Nutrition* **54,** *461 (1954)*; **65,** *161 (1958)*; *Brit. J. Nutrition* **9,** *340 (1955)*; *abstracts of meeting of American Chemical Society, Chicago 1953, p. 26A; Fed. Proc.* **13,** *476 (1954)*; **17,** *483 (1958)*).

While this increased amount of thiamine was found to be made available to the rat by coprophagy in the case of thiamine (*Fed. Proc.* **18,** *550 (1959)*; *J. Nutrition* **69,** *81 (1959)*), in the case of pantothenic acid deficiency and antibiotic response, the increased response was found not to be related to coprophagy since the pantothenic acid formed was directly available to the animal (*J. Nutrition* **69,** *81 (1959)*).

B. Connor Johnson
Urbana, Illinois

Nitrogen Balance and Exchangeable Albumin Pool

Since the protein metabolism of the undernourished or postoperative patient is dependent upon many factors such as nutritional intake, infection or actual loss of protein from the body, one can obtain the best overall index as to what is actually occurring by applying as many different methods to the same problem as possible. Recently R. E. Madden (*Lancet* **II,** *939 (1959)*) studied the nitrogen balance in three undernourished patients before and after seven days of refeeding with a high-protein liquid diet. Two of the patients had upper gastrointestinal malignancies and were severely cachectic and the third had carcinomatosis. In addition to carrying out nitrogen balance studies, Madden measured the total exchangeable albumin pool both before and after the seven days of refeeding by using radioactive iodinated albumin.

It was found that the three subjects had positive protein balances of 259 to 371 g. for

the seven-day refeeding period, indicating that they were synthesizing large amounts of protein during this period. However, no significant change resulted in the size of the albumin pool expressed in grams. The changes in the pool ranged from 9.2 g. to −6.5 g. In the two patients with malignant gastric disease the half-life of the albumin was 5.3 and 8.2 days, while the half-life of the albumin in the patient with carcinomatosis was 16 days.

It seems unlikely that the patients could accumulate between 371 and 259 g. of protein without any change in the serum albumin level, unless all the protein was used for tissue or muscle. This discrepancy might be explained by the loss of large amounts of the tracer in the bowel, a condition which has been reported to occur in patients originally believed to have hypercatabolic hypoproteinemia (*Nutrition Reviews* **17,** *261* (*1959*)). This might also explain the short half-lives of the albumin noted in two of these individuals.

This investigator concludes that the total of exchangeable albumin pool measured in these three hypoproteinemic patients did not show significant increases in size after refeeding on a high-protein, high-calorie diet, in spite of positive protein balances of 40 to 50 g. per day. Such a study is of interest and should be repeated using polyvinylpyrrolidone tagged with radioactive iodine to show any possible losses of protein into the gut. Under such conditions it might be possible to seek out discrepancies between the two methods and arrive at a physiological explanation compatible with all procedures.

Recent Books

The Low Sodium, Fat-Controlled Cookbook. By Alma Smith Payne and Dorothy Callahan. Published by Little Brown & Co., Boston, Mass. Pp. 429. Price $4.75.

The Merck Index of Chemicals and Drugs, Seventh Edition. An encyclopedia for chemists, pharmacists, physicians and members of allied professions. Published by Merck & Co., Rahway, New Jersey. 1960. Pp. 1641.

Modern Nutrition in Health and Disease. Second Edition. Edited by Michael G. Wohl and Robert S. Goodhart. Lea & Febiger, Philadelphia, Pa. 1960. Pp. 1152. Price $18.50.

Foods Without Fads. E. W. McHenry, University of Toronto. J. B. Lippincott Co., Philadelphia and New York. 1960. Pp. 159. Price $3.50.

Nutrition in Clinical Dentistry. Abraham E. Nizel. W. B. Saunders Co., Philadelphia and London. 1960. Pp. 467. Price $10.00.

New Jersey State Diet Manual. Project of Diet Therapy Section, New Jersey Dietetic Association, 1958–1959. Published by New Jersey State Department of Health. Pp. 84.

Losses due to Agricultural Pests. Summary of Conference of the Agricultural Board Committee on Agricultural Pests, November 1959. National Academy of Sciences-National Research Council. Pp. 20.

The Measurement of Grassland Productivity. Edited by Professor J. D. Ivins. Academic Press, Inc., New York. 1959. Pp. 212. Price $7.00.

Essentials of Healthier Living. By Justus J. Schifferes, Director, Health Education Council. Published by John Wiley & Sons, Inc., New York, N. Y. Pp. 327. Price $5.50.

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